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Evaluation of Phytochemical Contents and *In Vitro* Antioxidant, Anti-Inflammatory, and Anti-Acne Activities of Aqueous Extracts from A Traditional Thai Polyherbal "Ya Mong Ngoo" Formulation

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Abstract

This study aimed to evaluate the biological potential of aqueous extracts from herbal ingredients in the traditional Ya Mor Ngu formulation, namely *Angiopteris evecta* (G. Forst.) Hoffm., *Clinacanthus nutans* (Burm.f.) Lindau, *Tacca chantrieri André*, and *Gomphrena globosa L.* as well as to develop an herbal serum formulation for skincare applications. All herbal materials were subjected to quality control by determination of moisture content according to AOAC methods prior to aqueous extraction and freeze drying. Subsequently, the extracts were evaluated for antioxidant activities (DPPH and ABTS assays), total phenolic content (TPC), total flavonoid content (TFC), *in vitro* anti-inflammatory activity (albumin denaturation assay), inhibitory activity against *Cutibacterium acnes* (*C. acnes*), and cytotoxicity toward human dermal fibroblasts using the SRB assay. The results demonstrated that all herbal raw materials had moisture contents below 10%. The extract of *Tacca chantrieri André*, exhibited the most prominent biological potential, showing strong antioxidant activities (DPPH and ABTS), which correlated with high total phenolic (TPC) and total flavonoid (TFC) contents, and it was the only extract capable of inhibiting *C. acnes*. In contrast, the extract of *Angiopteris evecta* (G. Forst.) Hoffm. showed superior anti-inflammatory activity. Cytotoxicity assessment at the cellular level confirmed the safety of all extracts at low to moderate concentrations. Regarding herbal serum development, the mixed extract formulation exhibited higher anti-inflammatory activity than the single extract formulation, whereas the serum containing a single extract demonstrated stronger inhibitory activity against *C. acnes* than the mixed formulation. Nevertheless, the *C. acnes* inhibitory activity of the serum formulations remained lower than that of the standard drug. In conclusion, extracts from the Ya Mor Ngu formulation, particularly *Tacca chantrieri André*, show potential for development as active ingredients in skincare products targeting antioxidant, anti-acne, and anti-inflammatory effects

Keywords: Ya Mong Ngoo, Polyherbal formulation, Aqueous extraction, Phytochemical contents, Antioxidant activity, Anti-Inflammatory activity, Anti-Acne activity

Introduction

Acne and cutaneous inflammation remain major concerns in both dermatological and cosmetic sciences. Acne is a multifactorial condition involving dysregulation of sebum production, inflammatory responses, oxidative stress, and the proliferation of *C. acnes*, a key microorganism that triggers inflammation within the pilosebaceous unit and contributes to acne lesion formation, thereby significantly affecting quality of life [1,2]. These complex pathogenic mechanisms have stimulated growing interest in natural bioactive agents capable of simultaneously reducing oxidative stress, suppressing inflammation, and inhibiting acne-associated microorganisms through multi-target

mechanisms. Consequently, such agents have become an important focus in functional cosmeceutical research and in the development of herbal-based products supported by scientific evidence [3,4].

Over the past decade, herbal skincare products have gained increasing popularity as consumers prioritize mildness, safety, and sustainability. Nevertheless, the assumption that natural products are inherently safe or effective is not always valid. Plant extracts may vary considerably in chemical composition depending on botanical origin, preparation methods, and extraction processes, while inadequate quality control may lead to contamination or skin irritation [3,5,6]. Therefore, integrating traditional knowledge with systematic scientific validation is essential to elevate traditional herbal formulations toward cosmeceutical applications that meet internationally accepted standards. The traditional Thai polyherbal formulation known as Ya Mong Ngoo, originating from Ban Suan Khanun community, Khao Lan Subdistrict, Thap Sakae District, Prachuap Khiri Khan Province, has been practiced since 1939. Historically, it has been used for the management of snake envenomation and skin inflammation through a holistic therapeutic approach. Several plants within this formulation demonstrate considerable potential for dermatological and cosmeceutical applications due to reported antioxidant, anti-inflammatory, and antimicrobial activities, which correspond to key mechanisms involved in acne pathogenesis. Polyherbal formulations are particularly valuable because diverse phytochemical constituents may produce synergistic or additive biological effects, providing multifunctional efficacy within a single formulation.

Previous ethnopharmacological and pharmacological studies have reported relevant bioactivities for individual plants within the Ya Mong Ngoo formulation. *Angiopteris evecta* (G. Forst.) Hoffm. has been traditionally used for treating skin disorders and inflammatory conditions across several cultures, illustrating ethnopharmacological convergence that supports the presence of genuine bioactive compounds rather than placebo-based effects [7]. *Clinacanthus nutans* (Burm.f.) Lindau contains compounds associated with anti-inflammatory and antimicrobial activities, partly through modulation of inflammatory pathways such as cyclooxygenase-2 and cytokine expression [8,9]. *Tacca chantrieri André* is known to contain saponins, flavonoids, and phenolic compounds with reported antioxidant and antimicrobial activities [10,11]. In addition, *Gomphrena globosa L.* contains natural pigments such as betacyanins and flavonoids that may contribute to antioxidant activity and protection against oxidative stress in the skin [12]. From a technological perspective, this study emphasizes aqueous extraction, which aligns with traditional Thai medicine practices and community-based preparation methods. To improve reproducibility and extract standardization, controlled high-temperature aqueous extraction followed by freeze-drying was applied to preserve bioactive constituents and produce stable dry extract powders suitable for cosmeceutical formulation development [13]. This approach represents a translational strategy bridging traditional herbal preparations and standardized raw materials for modern product development.

Accordingly, the present study aims to systematically evaluate the phytochemical profiles and *in vitro* biological activities of aqueous extracts derived from key herbal components of the traditional Ya Mong Ngoo formulation. The investigation includes (1) Determination of total phenolic and flavonoid contents, (2) Assessment of antioxidant activity using DPPH and ABTS assays, (3) Evaluation of anti-inflammatory activity using a protein denaturation model, and (4) Preliminary assessment of anti-acne activity via inhibition of *C. acnes.* In addition, the study extends beyond extract level evaluation by developing prototype herbal serum formulations to explore functional applicability in cosmeceutical systems. Despite increasing research on individual herbal extracts and polyherbal systems, limited studies have comprehensively integrated phytochemical characterization, multi-functional biological evaluation, and formulation development within a single framework, particularly for traditional Thai formulations. Therefore, this study presents 3 key novel contributions: (1) the scientific validation of a traditional Thai polyherbal formulation using a standardized aqueous extraction approach that reflects traditional preparation methods; (2) the integrative evaluation of multiple biological activities relevant to acne pathogenesis; and (3) the translation of these findings into prototype formulations, bridging

traditional knowledge and modern cosmeceutical development.

Overall, this work provides a systematic and translational framework for advancing traditional herbal formulations toward evidence-based cosmeceutical applications aligned with international standards.

Materials and methods

Materials

Plant materials; the herbal materials used in this study were obtained from the Ya Mor Ngu traditional formulation based on local wisdom of the Ban Suan Khanun community, Khao Lan Subdistrict, Thap Sakae District, Prachuap Khiri Khan Province, Thailand. The formulation comprised 4 herbal species: *Angiopteris evecta* (G. Forst.) Hoffm., using the rhizomes; *Clinacanthus nutans* (Burm.f.) Lindau, using the leaves; *Tacca chantrieri André*, using the rhizomes; and *Gomphrena globosa L.* (purple globe amaranth), using the flowers and flower stalks. The plant samples were prepared as voucher specimens and deposited at the Thai Pharmacy laboratory, Department of Public Health, Faculty of Science and Technology, Chiang Mai Rajabhat University. All specimens were examined and taxonomically authenticated by Supat Lhanyanai, a botanist from the Thai Traditional Medicine Program, Department of Public Health, Faculty of Science and Technology, Chiang Mai Rajabhat University. The voucher specimen numbers were as follows: TMCMRU09 (*Clinacanthus nutans* (Burm.f.) Lindau), TMCMRU10 (*Tacca chantrieri André*), TMCMRU11 (*Gomphrena globosa L.*; Purple globe amaranth), and TMCMRU12 (*Angiopteris evecta* (G. Forst.) Hoffm.). All plant materials were thoroughly cleaned, dried, and processed into herbal powders according to the procedures described in the materials and methods section.

Cell line and microorganism Human skin fibroblasts at passage 56 were kindly provided by the Faculty of Medicine, Chiang Mai University, and were used as an *in vitro* model for the evaluation of cytotoxicity at the cellular level. *C. acnes* was kindly provided by the Faculty of Medicine, Chiang Mai University, and was used for the assessment of antibacterial activity related to acne-associated infection.

Chemicals and reagents; the chemicals and reference standards used in this study included sodium lauryl sulfate (SLS) as a standard irritant, clindamycin (2 µg/disc) (Oxide Ltd., UK), diclofenac diethylammonium (Difelene®, Thailand), Trolox, and ascorbic acid (Sigma-Aldrich, USA). Cell culture media and biochemical reagents consisted of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (HiMedia Laboratories Pvt. Ltd., India) and 1% penicillin/streptomycin, phosphate-buffered saline (PBS, pH 7.4), and trypsin-EDTA. Reagents used for cytotoxicity testing included trichloroacetic acid (TCA), sulforhodamine B (SRB) 0.4%, acetic acid, and Tris base. Reagents for biological activity assays included DPPH and ABTS (Sigma-Aldrich, USA). Ingredients used for formulation preparation included hydroxypropyl methylcellulose (HPMC, 50 cP), sodium hyaluronate, propylene glycol, glycerin, sodium olivate, phenoxyethanol, fragrance, 95% ethanol (AR grade), and distilled water (My Skin Recipes, Thailand).

The laboratory equipment used in this study included a pH meter (Seven Compact™ S220; Mettler-Toledo, Switzerland), hot air oven (Memmert UN series; Memmert GmbH, Germany), analytical balance (Entris II BCE series; Sartorius, Germany; readability 0.1 mg), CO_2 incubator (Heracell™ VIOS series; Thermo Scientific, USA; 37 °C, 5% CO_2), biosafety cabinet (Class II Type A2; Esco Airstream®, Esco Lifesciences, Singapore), inverted microscope (CKX53; Olympus, Japan), microplate reader (Varioskan™LUX; Thermo Scientific, USA), and a UV-Visible spectrophotometer (laboratory-grade UV-Vis spectrophotometer; Analytik Jena, Germany). Other general laboratory equipment included a water bath/shaking water bath, plate shaker, micropipettes, sterile 96-well microplates, culture flasks, centrifuge tubes, and filter paper discs (6 mm in diameter).

Methods

Preparation of herbal raw materials

Samples of the 4 herbal species; *Angiopteris evecta* (G. Forst.) Hoffm., *Clinacanthus nutans* (Burm.f.) Lindau, *Tacca chantrieri André*, and *Gomphrena globosa L.* (purple globe amaranth) were collected from the Ban Suan Khanun community. The

plant materials were washed twice with clean water, air-dried in the shade for 2 days, and subsequently oven-dried at 50 °C until the moisture content was < 10%, in accordance with AOAC criteria [14] (**Figure 1**). Moisture content was determined according to AOAC standards [14]. Briefly, containers were dried at 105 °C, cooled in a desiccator, and weighed until a constant weight was achieved. Approximately 3 g of each sample was weighed and dried at 105 °C for 3 - 6 h, followed by cooling and reweighing until a constant weight was obtained (weight difference not exceeding 1 - 3 mg). Moisture content was calculated using Eq. (1):

$$\text{Moisture (\%)} = [(W1 - W2)\times 100]/W1 \quad (1)$$

where W1 is the weight before drying and W2 is the weight after drying. The dried herbal materials were ground and sieved through a No. 40 mesh (0.425 mm) to obtain a uniform powder. The resulting powders were then weighed, and the weights were recorded as the dry raw material weights.

(a) (b) (c) (d)

Figure 1 Dried herbal raw materials used in the study: *Angiopteris evecta* (G. Forst.) Hoffm. rhizome (a), *Clinacanthus nutans* (Burm.f.) Lindau leaves (b), *Tacca chantrieri André* rhizome (c), and *Gomphrena globosa L.* flowers (d). All samples were dried to a moisture content below 10% prior to extraction in accordance with AOAC guidelines [14].

Extraction

Reflux extraction was employed to reflect the traditional herbal decoction practice of local wisdom. Accordingly, the aqueous extraction was chosen to mimic traditional herbal decoction and to efficiently extract polar phytochemicals associated with the biological activities of the formulation [13]. The herbal powders were extracted with distilled water at a ratio of 1:10 (w/v) at 95 ± 2 °C for 1 h, followed by a standing period of 1 h. The extracts were then filtered, and the filtrates were freeze dried overnight to obtain crude extracts in powder form. Four extracts were obtained and coded as follows: WFD (*Angiopteris evecta* (G. Forst.) Hoffm. rhizome extract), SFD (*Clinacanthus nutans* (Burm.f.) Lindau leaf extract), NFD (*Tacca chantrieri André* rhizome extract), and BFD (*Gomphrena globosa L.* flower extract). The percentage yield was subsequently calculated using Eq. (2).

$$\%\ \text{yield} = (\text{weight of crude extract (g)}/\text{weight of initial herbal material (g)})\times 100 \quad (2)$$

In vitro biological activity evaluation

(1) Antioxidant activity assays: DPPH assay [15]; A 0.2 mM DPPH solution was prepared in ethanol. The herbal extracts were prepared at 5 different concentrations, with ascorbic acid used as a reference standard. Each sample was mixed with the DPPH solution and incubated in the dark for 30 min. The absorbance was measured at 517 nm. The percentage of radical scavenging activity (% inhibition) was calculated using Eq. (3), and the IC_{50} value was determined from the concentration, % inhibition curve (performed in triplicate, n = 3; results expressed as mean ± SD).

ABTS assay [17]; The $ABTS^{\cdot+}$ radical cation was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$) at a ratio of 2:1 and incubating the mixture in the dark for 16 h. The solution was then diluted with distilled water to obtain an absorbance of 0.70 ± 0.05 at 734 nm. A volume of 5 µL of sample or Trolox standard was mixed with 195 µL of $ABTS^{\cdot+}$ solution and incubated in the dark for 6 min. The absorbance was measured at 734 nm. The percentage inhibition was calculated using Eq. (3), and

the IC_{50} value was determined in the same manner as described above (n = 3).

$$\text{Inhibition activity (\%)} = [(A_{blank} - A_{sample})/A_{blank}] \times 100 \quad (3)$$

where A_{blank} is the absorbance of the control solution and A_{sample} is the absorbance of the solution containing the test sample.

(2) Quantitative phytochemical determination: Total flavonoid content (TFC) [16] was determined using catechin as a reference standard (20 - 100 µg/mL). Herbal extracts were prepared at a concentration of 1 mg/mL in ethanol. A 2% $AlCl_3 \cdot 6H_2O$ solution was added, and the absorbance was measured at 510 nm. TFC was calculated from the catechin standard calibration curve (n = 3).

Total phenolic content (TPC) [16] was determined using gallic acid as a reference standard. Herbal extracts were prepared at a concentration of 1 mg/mL and mixed with Folin-Ciocalteu reagent, followed by incubation for 3 min. Subsequently, 7.5% Na_2CO_3 was added, and the mixture was incubated for 15 min. The absorbance was measured at 765 nm. TPC was calculated using Eq. (4) and expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract) (n = 3).

$$C = (c \times V)/\text{m} \quad (4)$$

where C is the total phenolic content, c is the concentration obtained from the standard calibration curve, V is the volume of the extract, and m is the weight of the extract.

(3) Anti-inflammatory activity (inhibition of albumin denaturation): The anti-inflammatory activity of the extracts was preliminarily evaluated using an albumin denaturation assay based on a heat-induced protein denaturation model (ovalbumin model) [13,17]. This method assesses the ability of test samples to inhibit protein denaturation, which is associated with inflammatory processes. Test samples and the reference anti-inflammatory drug diclofenac diethylammonium were dissolved in distilled water and serially diluted to final concentrations of 0.625, 1.25, 2.5, 5, and 10 mg/mL. Each prepared sample solution was incubated with albumin and subjected to heat treatment at 70 ± 2 °C for 5 min to induce protein denaturation. After incubation, absorbance was measured to determine the extent of albumin denaturation relative to the reference control. The concentration required to inhibit albumin denaturation by 50% (IC_{50}) was subsequently calculated using Eq. (5).

$$\text{Inhibition activity (\%)} = [(A_{blank} - A_{sample})/A_{blank}] \times 100 \quad (5)$$

where A_{blank} is the absorbance of the control solution and A_{sample} is the absorbance of the solution containing the test sample.

(4) Inhibitory activity against *C. acnes* (disc diffusion assay) [18]: Test samples were dissolved in distilled water. WFD showed a maximum solubility of 100 mg/mL, whereas BFD, NFD, and SFD showed maximum solubility of 200 mg/mL. All solutions were sterilized by filtration through a 0.2 µm membrane filter. WFD was aseptically prepared at concentrations of 1, 10, and 100 mg/mL, while BFD, NFD, and SFD were aseptically prepared at concentrations of 2, 20, and 200 mg/mL. The antibacterial activity against *C. acnes* was evaluated using the disc diffusion method. For each test, 10 µL of sample solution was applied onto a 6 mm diameter filter paper disc. This resulted in applied sample amounts of 0.01, 0.1, and 1 mg for WFD, and 0.02, 0.2, and 2 mg for BFD, NFD, and SFD. Clindamycin (0.002 mg/disc) was used as the positive control, while the solvent used to dissolve the samples served as the negative control. A bacterial suspension of *C. acnes* adjusted to 0.5 McFarland standard was uniformly spread over the surface of brain heart infusion (BHI) agar using a cotton swab. The prepared filter paper discs were then placed onto the agar surface. The plates were incubated at 37 ± 1 °C for 48 h under anaerobic conditions. Antibacterial activity was assessed by measuring the diameter of the inhibition zones in millimeters (mm). The disc diffusion assay was employed as a preliminary screening method for evaluating antibacterial activity against *C. acnes.* It should be noted that diffusion-based assays are influenced by the ability of compounds to diffuse through agar media and therefore provide only preliminary information on antibacterial potential.

(5) Cytotoxicity assay (SRB assay): Cell culture and maintenance; Human dermal fibroblast cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin under standard culture conditions (37 °C, 5% CO_2). Cells were subcultured using standard trypsinization procedures and routinely monitored for morphology and growth characteristics using phase-contrast microscopy. All experiments were conducted using cells within the same passage range to minimize experimental variability. Prior to each experiment, cell morphology and viability were examined to ensure the maintenance of normal fibroblast characteristics. Cytotoxicity of the extracts toward human dermal fibroblasts was evaluated using the sulforhodamine B (SRB) assay according to previously described methods [19]. Briefly, fibroblast cells were seeded into 96-well plates at a density of 5×10^3 cells per well and allowed to attach for 24 h. The cells were then treated with the test samples at various concentrations, with the solvent concentration kept constant across all wells, and further incubated for 48 h. Cells were fixed with cold 10% trichloroacetic acid (TCA) and stained with 0.4% SRB in 1% acetic acid for 30 min. Excess dye was removed by washing with 1% acetic acid 3 - 4 times, and the plates were air-dried. The bound dye was subsequently solubilized with 10 mM Tris base (pH 10.5), shaken for 10 - 20 min, and the absorbance was measured at 540 - 65 nm. Cell viability (%) was calculated using Eq. (6), and the IC_{50} value was determined from the dose response curve using non-linear regression analysis. Before each experiment, cell cultures were examined under a microscope to confirm normal morphology and adherence. Only cultures exhibiting typical fibroblast characteristics and good viability were used for subsequent experiments.

$$\% \text{ Cell viability} = (\text{Asample}\times100)/\text{Acontrol} \quad (6)$$

where $A_{control}$ is the absorbance of the untreated control cells and A_{sample} is the absorbance of cells treated with the test sample.

Development of herbal serum and product evaluation

The basic serum formulation was prepared based on a modified formulation guideline provided by cosmetic raw material manufacturers. The effect of thickening agents on the physical properties of the serum was investigated. The formulation process was conducted under heating conditions, with the temperature controlled between 70 - 80 °C during the mixing of the main components. The composition of the basic serum formulation is presented in **Table 1**.

Table 1 Composition of the base serum formulation.

No.	Chemical ingredients	Amount (% w/w)	Function
1	Aqua	Qs to 100	Diluent
2	Hydroxypropyl methyl cellulose	0.30	Thickener
3	Hydroxypropyl ethyl cellulose	0.70	Thickener
4	Sodium hyaluronate	0.10	moisturizer
5	Propylene glycol	2.00	Humectant
6	Glycerin	3.00	Humectant
7	Phenoxyethanol	1.00	Preservative
8	Fragrance	0.10	Fragrance

A small amount of fragrance (0.10% (w/w)) was incorporated into the base serum formulation to improve sensory acceptability. Although fragrance compounds may exhibit minor biological activities in some cases, the concentration used in this study was kept low and identical across all formulations to minimize potential interference with biological activity evaluations.

Preparation of mixed extract serum formulations; The selection of the mixed extract ratio (50:20:20:10,

% (w/w)) was based on the relative ranking of biological activities observed among the individual extracts, including antioxidant activity (DPPH and ABTS), anti-inflammatory activity, antibacterial activity against *C. acnes,* and cytotoxicity profiles. Extracts exhibiting stronger overall biological performance were assigned higher proportions in the formulation. Accordingly, the extract demonstrating the highest integrated biological potential (NFD) was incorporated at the highest proportion, followed by the second to fourth ranked extracts. This semi-quantitative approach was adopted as a preliminary formulation strategy to explore potential synergistic interactions among the herbal extracts. The mixed extracts were incorporated into the base serum at total concentrations of 1% (w/w), 3% (w/w), and 5% (w/w), resulting in formulations SF1, SF2, and SF3, respectively. In addition, single extract serum formulations containing only the highest ranked extract were prepared at the same concentrations (1% (w/w), 3% (w/w), and 5% (w/w)) and designated as SFN1, SFN2, and SFN3. In total, 6 serum formulations were developed. The extracts were added during the final formulation step when the serum temperature had decreased to 40 - 45 °C and mixed at a constant speed for 1 min to minimize thermal degradation of bioactive compounds. The selected extract concentrations (1% (w/w) - 5% (w/w)) were used to investigate dose response relationships within a practical cosmetic application range, supported by previous reports on the use of plant extracts in topical anti-inflammatory and anti-acne formulations at low to moderate concentrations [20].

All 6 serum formulations were evaluated for product-related biological activities, including *in vitro* anti-inflammatory activity and *C. acnes* inhibitory activity. For the *in vitro* anti-inflammatory activity, the assay was conducted by dissolving the test serum samples and the reference anti-inflammatory drug diclofenac diethylammonium in distilled water. The sample solutions were diluted to final concentrations of 0.625, 1.25, 2.5, 5, and 10 mg/mL and then incubated with albumin at an elevated temperature (70 ± 2 °C) for 5 min. The absorbance was subsequently measured to determine the extent of albumin denaturation in comparison with the reference standard. The concentration required to inhibit albumin denaturation by 50% (IC_{50}) was determined following the previously reported method [22]. For the *C. acnes* inhibitory activity, serum samples were sterilized by filtration through a 0.2 µm membrane filter to obtain the undiluted preparation. A stock solution was prepared by dissolving 1,000 mg of serum in 1 mL of distilled water, followed by aseptic dilution to obtain concentrations of 10, 100, and 1,000 mg/mL. Antibacterial activity against *C. acnes* was evaluated using the disc diffusion method. For each test, 10 µL of sample solution was applied onto a 6 mm diameter filter paper disc, resulting in applied sample amounts of 0.1, 1, and 10 mg per disc, as well as the undiluted sample. Clindamycin (0.002 mg/disc) was used as the positive control, while the solvent used to dissolve the samples served as the negative control. A *C. acnes* suspension adjusted to 0.5 McFarland standard was uniformly spread over the surface of brain heart infusion (BHI) agar using a cotton swab. The prepared filter paper discs were placed onto the agar surface, and the plates were incubated at 37 ± 1 °C for 48 h under anaerobic conditions. Antibacterial activity was assessed by measuring the diameter of the inhibition zones in millimeters (mm).

Quality control of herbal extracts

All herbal extracts were prepared as a single production batch to minimize batch-to-batch variability. After freeze drying, the extracts were stored in airtight, light-protected containers at −20 °C until use, while repeated freeze - thaw cycles were avoided. Prior to biological activity evaluation, all extracts were subjected to preliminary quality control assessments, including: (1) physical characteristics (color, odor, and powder appearance), (2) re-solubility in the solvents used for the assays, and (3) stability of the extracts under storage conditions throughout the experimental period. Extracts exhibiting significant physical changes or showing precipitation or discoloration after reconstitution were excluded from biological testing to ensure that the experimental results reflected the activity of extracts with adequate quality and consistency.

Quality control and uniformity of serum formulations

All serum formulations were prepared under identical conditions, with strict control of processing temperature, mixing time, and the sequence of ingredient addition. After preparation, each formulation was preliminarily evaluated for physical properties, including texture, homogeneity, color, odor, and pH, to confirm suitability for biological testing. Before anti-inflammatory and *C. acnes* inhibitory activity evaluations, all serum formulations were allowed to equilibrate at room temperature for at least 24 h to ensure system stabilization and to minimize the effects of initial post production instability.

Data and statistical analysis

All experiments were conducted with at least 3 independent replicates (n = 3), and results are expressed as mean ± standard deviation (SD). The concentrations producing 50% inhibitory effects (IC_{50}) were calculated using appropriate regression models. Statistical differences among groups were analyzed using 1-way analysis of variance (one-way ANOVA). When significant differences were detected, Tukey's post-hoc multiple comparison test was applied to determine differences between individual groups. Statistical significance was defined at $p < 0.05$.

Results and discussion

Results of herbal raw material preparation and moisture content quality control; the herbal materials used in this study were collected from the Ban Suan Khanun community, Khao Lan Subdistrict, Thap Sakae District, Prachuap Khiri Khan Province, Thailand. The samples were cleaned and dried using a hot air dehydrator, with temperature and drying time adjusted according to the characteristics of each raw material. Moisture content determination based on AOAC standards [14] revealed that all 4 herbal materials had moisture contents below 10%, namely *Angiopteris evecta* rhizomes (8.33%), *Clinacanthus nutans leaves* (5.67%), *Tacca chantrieri André* rhizomes (7.67%), and *Gomphrena globosa L.* flowers (5.33%). These results indicate that all raw materials met the acceptable criteria for subsequent extraction and biological activity evaluation.

Preparation of aqueous extracts and physical characteristics of crude extracts; Aqueous extraction was performed using distilled water as the solvent at herb to solvent ratio of 1:10 (w/v) at 95 ± 2 °C for 1 h, followed by a standing period of 1 h prior to filtration. The filtrates were then freeze dried to obtain crude extracts in dry powder form. The extraction yields (% yield) and physical characteristics of the crude extracts differed markedly among the herbal species. The WFD resulted in a light-brown powder, the SFD resulted in a dark-brown powder, the NFD resulted in a light-brown powder, and the BFD resulted in a dark-brown powder. The percentage yields of the respective extracts were 7.63%, 11.80%, 4.56%, and 6.56%, respectively (**Figure 2**).

(a) (b) (c) (d)

Figure 2 Freeze dried crude extracts obtained from aqueous extraction of herbal materials: WFD (*Angiopteris evecta* (G. Forst.) Hoffm. rhizome extract) (a), SFD (*Clinacanthus nutans* (Burm.f.) Lindau leaf extract) (b), NFD (*Tacca chantrieri André* rhizome extract) (c), and BFD (*Gomphrena globosa L.* flower extract) (d). Extraction was performed using distilled water (1:10 w/v) at 95 ± 2 °C for 1 h, followed by freeze drying to obtain stable powdered extracts.

The antioxidant activities of the herbal extracts were evaluated using DPPH and ABTS assays. Based on IC_{50} values (the concentration required to inhibit 50% of free radicals), the NFD extract exhibited the strongest antioxidant activity among the tested herbal extracts in the DPPH assay, with an IC_{50} value of 65.00

± 0.84 µg/mL. This was followed by WFD (505.26 ± 0.35 µg/mL), BFD (1,681.00 ± 0.70 µg/mL), and SFD (6,938.00 ± 0.75 µg/mL), respectively (**Table 2**). When compared with ascorbic acid, used as a reference standard (IC_{50} = 5.62 ± 0.20 µg/mL), the antioxidant activity of the NFD extract was significantly lower than that of the standard ($p < 0.05$). Nevertheless, the NFD extract showed significantly higher activity than the other herbal extracts ($p < 0.05$). Consistent trends were observed in the ABTS assay. The NFD extract again demonstrated the highest antioxidant activity, with an IC_{50} value of 23.00 ± 0.89 µg/mL, followed by BFD (92.00 ± 3.90 µg/mL), SFD (147.00 ± 6.77 µg/mL), and WFD (176.30 ± 17.49 µg/mL) (**Table 2**). Although the antioxidant activity of the NFD extract was lower than that of the Trolox standard (IC_{50} = 4.68 ± 0.16 µg/mL), it remained the most potent extract among all herbal samples tested.

Preliminary quantitative phytochemical analysis revealed marked differences in total flavonoid content (TFC) and total phenolic content (TPC) among the extracts. The NFD extract contained the highest levels of polyphenolic compounds in both parameters, with TFC and TPC values of 89.91 ± 0.20 mg/g extract and 138.41 ± 0.99 mg/g extract, respectively (**Table 2**). In contrast, the WFD extract showed TFC and TPC values of 8.97 ± 0.29 and 16.93 ± 0.53 mg/g extract, respectively, while the BFD extract exhibited TFC of 4.91 ± 0.25 mg/g extract and TPC of 23.89 ± 0.46 mg/g extract. The SFD extract presented TFC and TPC values of 4.44 ± 0.17 and 21.57 ± 0.27 mg/g extract, respectively. These results clearly demonstrate substantial differences in phytochemical composition among the different herbal species.

Table 2 Antioxidant activities and phytochemical contents of the 4 herbal extracts.

Samples	IC_{50} (µg/mL)		Total flavonoids (mg/g extract)	Total phenolic (mg/g extract)
	DPPH	ABTS		
NFD	65.00 ± 0.84[a]	23.00 ± 0.89[a]	89.91 ± 0.20[a]	138.41 ± 0.99[a]
WFD	505.26 ± 0.35[b]	176.30 ± 17.49[c]	8.97 ± 0.29[b]	16.93 ± 0.53[c]
BFD	1,681.00 ± 0.70[c]	92.00 ± 3.90[b]	4.91 ± 0.25[c]	23.89 ± 0.46[b]
SFD	6,938.00 ± 0.75[d]	147.00 ± 6.77[c]	4.44 ± 0.17[c]	21.57 ± 0.27[c]
Ascorbic acid (Std.)	5.62 ± 0.20	-	-	-
Trolox (Std.)		4.68 ± 0.16	-	-

Note: Values are expressed as mean ± SD (n = 3). Different superscript letters within the same column indicate statistically significant differences according to Tukey's post hoc test ($p < 0.05$).

The results of the *in vitro* anti-inflammatory assay based on albumin denaturation showed that the WFD and BFD extracts exhibited inhibitory activity, with IC_{50} values of 0.18 ± 0.00 mg/mL and 5.72 ± 0.13 mg/mL, respectively. The positive control, diclofenac diethylammonium, showed an IC_{50} value of 0.16 ± 0.02 mg/mL. Among the extracts, WFD exhibited an IC_{50} value most comparable to that of the reference drug. In contrast, the NFD and SFD extracts did not exhibit anti-inflammatory activity in this assay within the tested concentration range.

The results of the disc diffusion assay demonstrated that NFD exhibited inhibitory activity against *C. acnes* in the disc diffusion assay, suggesting potential antibacterial activity in this preliminary screening test. At a dose of 2 mg per disc, NFD produced an inhibition zone diameter of 11.34 ± 0.09 mm, whereas no inhibitory activity was observed at lower doses (0.2 and 0.02 mg). The WFD, BFD, and SFD extracts did not exhibit inhibitory activity at any of the tested concentrations (**Figure 3**). In comparison, clindamycin (0.002 mg/disc) produced a markedly larger inhibition zone of 48.57 ± 0.31 mm, indicating substantially higher antibacterial efficacy of the standard drug.

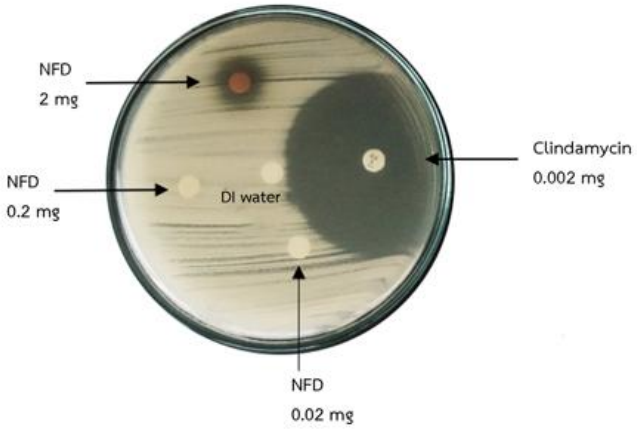


Figure 3 Inhibitory activity of NFD extract against *C. acnes* evaluated using the disc diffusion assay. Samples were applied at different concentrations onto 6 mm filter paper discs and incubated for 48 h under anaerobic conditions at 37 ± 1 °C. Antibacterial activity was expressed as inhibition zone diameter (mm). Clindamycin (0.002 mg/disc) was used as the positive control.

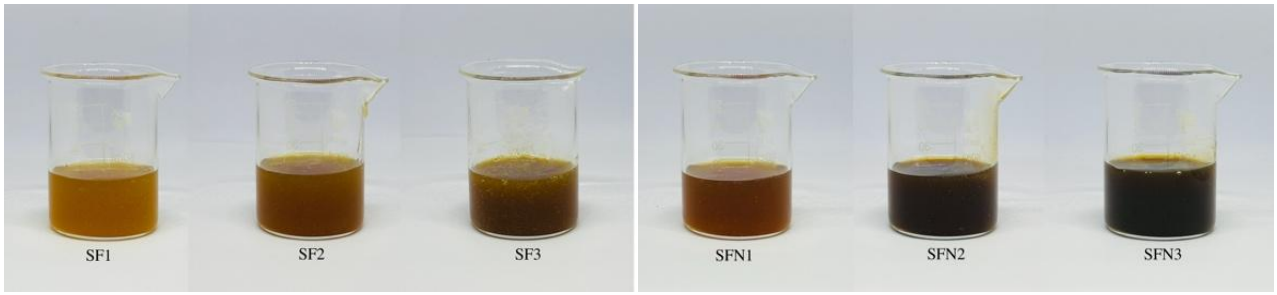
The cytotoxicity evaluation revealed that all 4 herbal extracts were safe to human dermal fibroblasts within the tested concentration ranges. The BFD, WFD, and NFD extracts at concentrations of 0.001 - 1 mg/mL resulted in cell viability ranges of 92.85% - 99.17%, 74.37% - 97.00%, and 87.66% - 97.71%, respectively. The SFD extract, tested at concentrations of 0.001 - 10 mg/mL, showed cell viability ranging from 87.14% - 98.73%. In contrast, sodium lauryl sulfate, used as the positive control, exhibited pronounced cytotoxicity, with cell viability markedly reduced to approximately 4.23 ± 0.54%, 3.78 ± 0.12%, and 3.05 ± 0.09% at concentrations of 0.1, 1, and 10 mg/mL, respectively (**Table 3**). These findings indicate that the investigated herbal extracts did not induce cytotoxic effects on human dermal fibroblasts within the tested concentration ranges, whereas the positive control demonstrated clear cytotoxicity.

Table 3 Percentage of viable human dermal fibroblasts following treatment with different concentrations of the samples.

Sample / Concentration (mg/mL)	**Cell viability (%)**				
	0.001	**0.01**	**0.1**	**1**	**10**
BFD	99.17 ± 4.54	98.95 ± 4.93	97.94 ± 3.87	92.85 ± 5.34	51.79 ± 3.85
WFD	97.00 ± 3.64	96.90 ± 3.78	94.48 ± 2.87	74.37 ± 3.60	16.36 ± 0.42
SFD	98.73 ± 1.85	97.52 ± 2.41	98.59 ± 1.34	97.50 ± 1.86	87.14 ± 2.08
NFD	97.71 ± 2.60	96.80 ± 2.66	96.00 ± 1.12	87.66 ± 1.12	35.11 ± 1.52
Sodium lauryl sulfate	96.31 ± 1.57	93.83 ± 0.86	4.23 ± 0.54	3.78 ± 0.12	3.05 ± 0.09

When evaluated integrative, NFD was the most suitable primary active ingredient, exhibiting the highest antioxidant activity (DPPH and ABTS), the greatest total polyphenol and flavonoid contents, exclusive inhibitory activity against *C. acnes* in the disc diffusion assay, and cellular safety within the tested concentration range. WFD, although less prominent in antioxidant and antimicrobial assays, showed anti-inflammatory activity comparable to the reference drug and is therefore appropriate as a complementary anti-inflammatory component. In contrast, BFD and SFD showed lower apparent activity

under the applied extraction conditions and assay systems, which should be interpreted as methodological limitations rather than a lack of intrinsic biological potential. Based on the overall ranking of the biological potential of the 4 herbal extracts, NFD exhibited the highest potential, followed by WFD, BFD, and SFD, respectively. Accordingly, a mixed extract formulation was developed using the extracts ranked first to fourth at a ratio of 50:20:20:10 (% w/w) and incorporated into the base serum at total extract concentrations of 1% (w/w), 3% (w/w), and 5% (w/w), resulting in formulations SF1, SF2, and SF3, respectively. In addition, single extract serum formulations containing NFD at the same concentrations were prepared and designated as SFN1, SFN2, and SFN3, yielding a total of 6 formulations for biological evaluation. Overall, the mixed extract serum formulations SF1-SF3 exhibited a golden-brown appearance with a pH of 5.5, good skin absorption, and a non-greasy texture (**Figure 4**), whereas the single extract serum formulations SFN1-SFN3 showed a dark-brown appearance with a pH of 6, similarly demonstrating good skin absorption and a non-greasy texture (**Figure 4**).



(a) (b)

Figure 4 Physical characteristics of herbal serum formulations: mixed extract formulations (SF1 - SF3) (a) and single extract formulations (SFN1 - SFN3) (b). All formulations were evaluated for appearance, color, homogeneity, and pH after stabilization at room temperature.

In the *in vitro* anti-inflammatory assay based on inhibition of albumin denaturation, the mixed extract formulations SF1, SF2, and SF3 exhibited anti-inflammatory activity, with SF2 showing the highest efficacy (IC_{50} = 9.89 ± 1.48 mg/mL). SF1 and SF3 showed IC_{50} values of 35.27 ± 7.51 and 31.75 ± 5.28 mg/mL, respectively. In contrast, the single extract NFD formulations (SFN1-SFN3) did not exhibit anti-inflammatory activity under the same experimental conditions. The reference drug diclofenac diethylammonium demonstrated markedly higher anti-inflammatory activity, with IC_{50} values in the range of 0.16 - 0.30 mg/mL.

Evaluation of inhibitory activity against *C. acnes* revealed that the mixed extract formulations (SF1 - SF3) and the single extract formulations SFN1-SFN2 did not exhibit antibacterial activity under the tested conditions. However, SFN3, containing 5% (w/w) NFD, exhibited low inhibitory activity against *C. acnes* when tested in the undiluted form, producing an inhibition zone diameter of 6.85 ± 0.11 mm. This activity was significantly lower than that of the standard drug clindamycin, which produced inhibition zones ranging from 48.44 to 49.95 mm (**Figure 5**).

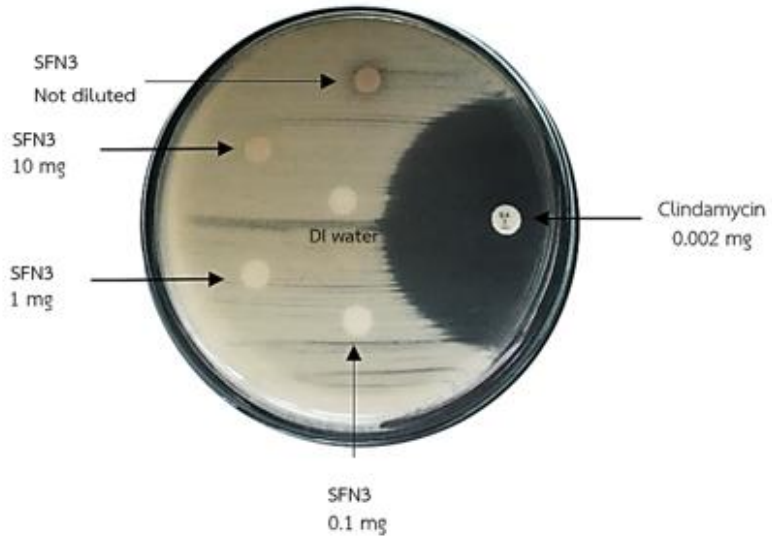


Figure 5 Inhibitory activity of the SFN3 serum formulation against *C. acnes* determined using the disc diffusion assay. The undiluted serum was applied onto 6 mm filter paper discs and incubated for 48 h under anaerobic conditions at 37 ± 1 °C. Antibacterial activity was expressed as inhibition zone diameter (mm) and compared with clindamycin (0.002 mg/disc) as the positive control.

Low moisture content (< 10%) confirmed the suitability of the herbal raw materials for extraction and ensured the stability of bioactive compounds during storage, consistent with AOAC standards [14]. Differences in extraction yield among the samples primarily reflected variations in water solubility rather than biological potency. This was evident from the strong biological activity of NFD despite its relatively low extraction yield, suggesting a higher proportion of active constituents in this extract. Among the tested samples, NFD consistently exhibited the strongest antioxidant activity in both DPPH and ABTS assays, which correlated with its markedly higher total phenolic and flavonoid contents. These findings support the well-established role of polyphenolic compounds in radical scavenging activity [21,22]. Nevertheless, all extracts showed lower antioxidant activity compared to reference standards, which is expected given the complexity of crude extracts containing both active and inactive components. In contrast, WFD demonstrated the strongest anti-inflammatory activity in the albumin denaturation assay, despite relatively low antioxidant capacity and phenolic content. This discrepancy suggests that anti-inflammatory activity may be attributed to non-polyphenolic constituents. These findings highlight that antioxidant and anti-inflammatory activities are not necessarily directly correlated and may involve distinct chemical and mechanistic pathways. The albumin denaturation assay serves as a preliminary indicator of anti-inflammatory potential by modeling protein stabilization rather than directly measuring inflammatory mediators at the cellular level. Therefore, the observed anti-inflammatory activity should be interpreted with caution. In this context, the present study relies on preliminary *in vitro* models, and more comprehensive mechanistic investigations are required to confirm the underlying biological effects and strengthen translational relevance. These should include evaluation of nitric oxide production, cyclooxygenase (COX) and lipoxygenase (LOX) inhibition, as well as modulation of pro-inflammatory cytokines. In addition, quantitative antimicrobial assays, such as determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), would further support a more robust biological characterization. With respect to antibacterial activity, only NFD exhibited inhibitory effects against *C. acnes* in the disc diffusion assay. This observation is consistent with previous reports that phytochemicals such as polyphenols and flavonoids may interfere with bacterial growth and

cellular processes [10,11]. However, the inhibition zone was markedly smaller than that of clindamycin, indicating substantially lower antibacterial potency. In addition, the disc diffusion method provides only qualitative information and is strongly influenced by compound diffusion through agar media. Therefore, inhibition zone size may not accurately reflect intrinsic antibacterial activity. Collectively, these findings suggest that the observed effects should be considered preliminary, as the extracts exhibit only weak antibacterial activity against *C. acnes* under the tested conditions and should not be interpreted as definitive evidence of anti-acne efficacy. Further evaluation using quantitative approaches, such as determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), is required to confirm antimicrobial potential. Cytotoxicity evaluation demonstrated that all extracts were non-toxic to human dermal fibroblasts within the tested concentration ranges, supporting their potential for topical application. However, the use of high passage fibroblast cells may introduce variability in cellular responses. Future studies should validate these findings using lower passage cells or additional skin-relevant models. The development of mixed extract serum formulations revealed that combined extracts enhanced anti-inflammatory activity compared to single extract formulations. Notably, the formulation containing 3% total extract (SF2) exhibited the highest activity, suggesting a potential synergistic effect and a non-linear dose response relationship. The extract ratio (50:20:20:10) was determined based on relative biological performance and represents a preliminary formulation strategy rather than a statistically optimized model. Future studies should employ design of experiments (DoE) or response surface methodology (RSM) to systematically optimize extract ratios and formulation parameters. In terms of antibacterial activity at the formulation level, the overall efficacy remained limited, with inhibition observed only in the high-concentration NFD formulation and remaining substantially lower than that of the standard drug. Therefore, the developed formulations are more appropriately positioned for applications targeting anti-inflammatory effects and skin balance rather than as primary antibacterial agents for acne treatment.

Overall, the findings indicate that NFD is the most promising candidate for antioxidant and preliminary antibacterial applications, while WFD serves as a complementary anti-inflammatory component. The combination of multiple extracts may enhance formulation-level efficacy, particularly for anti-inflammatory activity. However, further mechanistic studies and quantitative antimicrobial evaluations are required to strengthen the scientific evidence and support translation into cosmeceutical applications.

Conclusions

This study concludes that the preparation process of herbal raw materials from the traditional Ya Mor Ngu formulation was appropriate and met quality standards. Aqueous extraction followed by freeze drying yielded crude extracts with clearly distinct physical characteristics and extraction yields, highlighting that extraction yield does not necessarily correlate directly with biological potency. In terms of biological potential, the aqueous extract of *Tacca chantrieri André* rhizome (NFD) demonstrated the most prominent performance. NFD exhibited strong antioxidant activity in both DPPH and ABTS assays, consistent with its highest total polyphenol and flavonoid contents, and was the only extract capable of inhibiting *C. acnes* under the experimental conditions. In contrast, the rhizome extract of *Angiopteris evecta* (WFD) showed anti-inflammatory activity in the albumin denaturation model comparable to that of the reference drug, despite its lower antioxidant activity and lower total polyphenolic content. These findings underscore the diversity of bioactive compound classes among the herbs and the specificity of the assay systems employed. Cytotoxicity evaluation using human dermal fibroblasts confirmed that all herbal extracts were non-toxic within low to moderate concentration ranges, supporting their potential for further development as active ingredients in cosmetic and cosmeceutical products. The development of herbal serum formulations revealed that mixed extract formulations exhibited greater anti-inflammatory potential than single extract formulations, particularly those containing a moderate total extract concentration. This observation suggests a synergistic interaction among bioactive constituents derived from multiple

herbs, as well as a non-linear dose response relationship. However, the *C. acnes* inhibitory activity of the serum formulations remained limited when compared with the standard drug. Therefore, further antimicrobial investigations using quantitative methods, such as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays, are necessary to confirm the anti-acne potential of the extracts. In summary, this research highlights that the aqueous extract of *Tacca chantrieri André* rhizome possesses the highest integrated biological potential, while *Angiopteris evecta* rhizome represents an important complementary component for anti-inflammatory activity. The combination of multiple herbal extracts can enhance formulation-level efficacy. Future research employing formulation optimization strategies may further refine the extract ratio to maximize biological activity and overall product performance. These findings support the feasibility of developing skincare products aimed at reducing inflammation and restoring skin balance. Future investigations may also evaluate fragrance-free formulations to confirm that the observed biological activities are attributable solely to the herbal extracts. However, further studies using advanced cellular and molecular inflammatory models, together with detailed phytochemical characterization, are required to confirm the underlying mechanisms and strengthen the scientific basis for industrial application. Nevertheless, further studies involving in-depth phytochemical characterization and mechanistic investigations at the cellular and molecular levels are required to strengthen the scientific conclusions and enhance the potential for industrial application.

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Declaration of Generative AI in Scientific Writing

Disclosure Regarding AI-Assisted Writing The authors declare that generative AI software (ChatGPT and Gemini) was applied strictly for grammar correction and stylistic editing. The use of these technologies did not extend to content creation or qualitative/quantitative data interpretation. Consequently, the authors take full responsibility for the manuscript's content, ensuring that the work is free from AI-generated misinformation or hallucinations.

CRediT Author Statement

Payao Saytongsuk: Conceptualization, Methodology, Supervision, Validation, Resources, and Writing-original draft; **Supalak Fakkham:** Methodology, Validation, Resources, and Writing-review & editing; **Thanya Promsorn:** Validation, Resources, and Writing-review & editing.

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